Appendix A
Original Case Histories: Lessons Learned

Since the USEPA research studies in the 1980s and the first TREs performed to meet permit requirements, there have been significant advances in the development and refinement of TRE procedures. These advancements become apparent upon review of the original case histories published in the first edition of the TRE manual. The case histories have been revisited in this manual to note the lessons learned and new approaches that can be taken to conduct TREs.

Many lessons have been learned in applying TIE/TRE procedures to different types of effluents using a variety of freshwater and saltwater test species. Perhaps the most significant improvements in the methods since the original case histories were performed have been the development and application of methods to:

- Identify causes of short-term chronic toxicity to both freshwater and estuarine/marine species.
- Track sources of chronic toxicity that can not be readily characterized in the TIE.
- Characterize, identify, and confirm organophosphate insecticide toxicity.
- Characterize toxic metals using improved EDTA and sodium thiosulfate tests.
- Characterize surfactant toxicity using multiple TIE manipulations.
- Confirm toxicants by the correlation approach.

The use of some of these updated methods is described below using the original case histories as examples. The following summaries are intended to show how similar TREs can be performed more quickly, cost-effectively, and accurately using the current procedures. These summaries also portray the steps taken over the last 10 years to improve the TRE procedures.

Baltimore, Maryland

In January 1986, USEPA, in cooperation with the City of Baltimore, began the first research study to develop a pragmatic approach and methods for conducting TREs at WWTPs (Botts et al., 1987). The City's Patapsco WWTP was selected for this study because of evidence of acute and chronic effluent toxicity. In addition, USEPA was interested in conducting a TRE at an urban WWTP, like the Patapsco WWTP, which receives its influent from a wide range of industrial discharges. The objectives of the TRE were to characterize the WWTP's capability for treatment of toxicity, evaluate techniques to identify the specific components of toxicity, and assess methods to trace toxicity to its source(s).

The study results showed that the WWTP influent had significant acute and chronic toxicity as measured by *C. dubia* [(mean 48-hour LC50=2.6% and mean 7-day chronic value (ChV)=1.2%], *M. bahia* (mean 96-hour LC50=23%), and Microtox® (EC50=8%). Although significant toxicity reduction occurred through treatment, substantial toxicity remained. The 48-hour LC50 for *C. dubia* averaged 6.3% effluent. An evaluation of the WWTP operations indicated that treatment performance was not the major cause of effluent toxicity.

Results of the TIE showed that acute effluent toxicity was removed by passing effluent samples through a C18 SPE column. Recovery of toxicity in the 75 to 95% methanol/water eluates from the C18 column suggested that the toxicants were non-polar organic compounds with relatively high octanol-to-water partition coefficients. However, GC/MS analysis of the toxic non-polar organic fractions was not successful in identifying the specific nonpolar organic

toxicants. Additional testing showed that the toxicants sorbed onto suspended solids in the effluent. Solids greater than 0.2 µm were found to be the major toxic fraction.

TIE Procedure Update

Since this study, USEPA developed procedures for identifying non-polar organic toxicants (1993a). If non-polar organic toxicity is indicated in the Phase I of the TIE, the toxicant(s) can be isolated and concentrated to improve the chances of identification using GC/MS analysis. This approach has been helpful in identifying organophosphate insecticides as causes of effluent toxicity at some POTWs (see examples below and Appendix F).

An evaluation of wastewater samples from selected candidate industries was performed to determine the major contributors of refractory toxicity to the WWTP. An important goal of this study was to develop and evaluate methods for tracking sources of toxicity in POTWs. A protocol was designed to measure the toxicity remaining after treatment at the WWTP, which is the toxicity that passes through in the final effluent. The residual or "refractory" toxicity of five major industrial users of the WWTP was evaluated by treating wastewater samples in a bench-scale batch simulation of the WWTP activated sludge process. Microtox® results indicated that two of the five industries were contributing refractory toxicity to the WWTP. Results of *C. dubia* tests were inconclusive due to an interference in the treatment simulation. This interference appeared to be caused by residual toxicity in the RAS used in testing.

RTA Procedure Update

Biomass toxicity may be reduced by washing the RAS with buffer solutions or laboratory water. Alternatively, a surrogate biomass from a POTW with a similar type of biological treatment process may be obtained for testing. Details are presented in Section 5.

Hollywood, California

In the late 1980s and early 1990s, the USEPA laboratory in Duluth, Minnesota, tested several POTW effluents in the process of developing TIE procedures. One of these effluents was the City of Hollywood POTW, which exhibited acute toxicity to *C. dubia* (Amato et al., 1992).

TIE Phase I tests showed that treatment with a C18 SPE column was the only step that reduced effluent toxicity. Acute toxicity was recovered from the C18 column by eluting the column with methanol. Additional C18 SPE column tests performed on 16 effluent samples showed that toxicity was consistently eluted in the 80 and 85% methanol fractions, which suggested that the cause of toxicity was the same among the various samples. These results provided evidence that the toxicant(s) was a non-polar organic compound(s). Further concentration and separation of the toxic fractions was done, followed by confirmation GC/MS analyses of the fractions. Analysis of selected 80 and 85% methanol fractions by GC/MS found sufficient concentrations of the insecticide diazinon to account for the observed acute toxicity to *C. dubia.*

TIE Procedure Update

In recent TIE guidance, USEPA (1991 and 1993a) recommends adding a metabolic blocker, PBO, to toxic effluent samples or methanol eluates as a subsequent test for the presence of metabolically activated toxicants like organophosphate insecticides. PBO has been shown to block the acute toxicity of diazinon, parathion, methyl parathion, and malathion to cladocerans, but does not affect acute sensitivity to dichlorvos, chlorfenvinphos, and mevinphos (Ankley et al., 1991). A reduction in acute or chronic toxicity by the PBO addition together with toxicity removal by the C18 SPE column and concentration data can provide strong evidence for the presence of selected organophosphate insecticides.

In the confirmation step (USEPA, 1989b), three Phase III confirmation steps were used to confirm diazinon as a cause of effluent toxicity: toxicant correlation, mass balance, and additional species testing.

Toxicant correlation was evaluated by plotting effluent diazinon concentrations and effluent LC50 values as shown in Figure A-1. The correlation coefficient (r value) was significant and confirmed that, from sample to sample, diazinon was consistently the cause of acute effluent toxicity. Also, the intercept of the regression line at 100% effluent (0.325) was near the diazinon LC50 of 0.35 µg/L, which indicated that diazinon accounted for nearly all of the observed acute effluent toxicity.

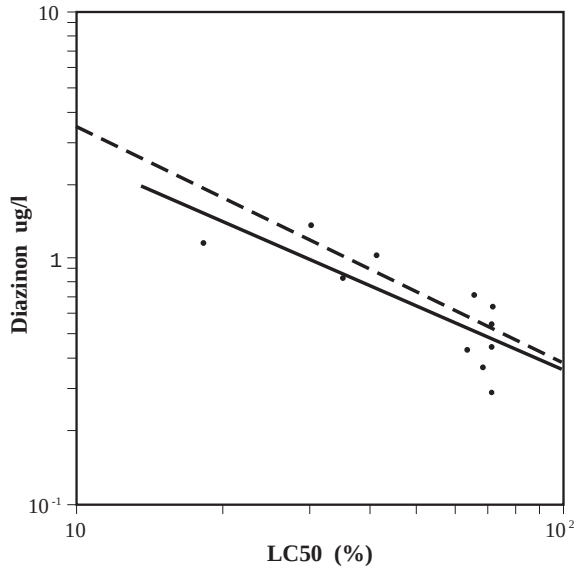

Figure A-1. Acute LC50 of Hollywood effluent versus diazinon concentration (actual correlation shown by solid line; predicted 1:1 correlation by dashed line) (Source: USEPA, 1988).

TIE Procedure Update

USEPA (1993b) recommends a straight-forward correlation approach to determine if a consistent relationship exists between the concentration of the toxicant(s) and effluent toxicity. This approach involves comparing the toxic units of the toxicant to whole effluent toxic units. Toxicant concentrations are converted to toxic units (i.e., measured concentration divided by the toxicant's acute or chronic endpoint) and the resulting values are plotted versus whole effluent toxic units. Since this study, additional acute toxicity data for diazinon and other organophosphate insecticides have become available for calculating toxic units for these toxicants (Ankley et al., 1991; Amato et al., 1992; and Bailey et al., 1997). The correlation approach is useful for determining the extent to which the identified toxicants contribute to effluent toxicity. Using the above example, diazinon would be confirmed as the primary toxicant if the slope is 1 and the intercept is 0 for a plot of diazinon toxic units versus effluent toxic units. In some cases, additional toxicants may be indicated using this technique (see the City of Largo, Florida, example below).

The mass balance confirmation approach involved passing samples through a C18 SPE column, eluting the column with a series of eight methanol concentrations, and testing the toxicity of the methanol fractions. The combined toxic, combined nontoxic, and all fractions were combined and tested at whole effluent concentrations. The results showed that the toxicity of the combined toxic fractions was similar to the toxicity of all fractions together and the toxicity of the original effluent samples. These results provided further confirmation that effluent toxicity was associated with non-polar organic toxicants.

The final confirmation step involved testing effluent samples with *P. promelas*, which are at least 100 times less acutely sensitive to diazinon than *C. dubia* (USEPA 1987, 1988). Test results showed only slight acute toxicity to the minnows as compared to the average acute LC50 of about 60% for *C. dubia*. Acute toxicity to *P. promelas* was interpreted as evidence that a toxicant other than diazinon was present in the samples. However, this additional toxicant(s) was not a significant contributor to toxicity and its identity was not evaluated. In summary, the Phase III testing confirmed that diazinon was the principal effluent toxicant.

Largo, Florida

The USEPA Duluth Laboratory also evaluated effluent samples from the City of Largo POTW. A TIE was performed to identify the causes of acute effluent toxicity (USEPA, 1987).

TIE Phase I tests showed that C18 SPE column treatment removed acute effluent toxicity. Toxicity was not reduced by the other Phase I treatments, including filtration, EDTA addition, or sodium thiosulfate addition.

An additional 18 effluent samples were passed through C18 SPE columns in Phase II. Elution of the columns with methanol showed that acute toxicity was consistently isolated in the 75 and 80% methanol concentrations, although occasional toxicity was also observed in the 70 and 85% methanol concentrations. GC/MS analysis of the toxic fractions identified diazinon as a cause of acute effluent toxicity.

In Phase III, five confirmation steps were used to verify that diazinon was the cause of effluent toxicity: toxicant correlation, toxicant spiking, mass balance, additional species testing, and test species symptoms.

Acute effluent toxicity and diazinon concentrations were converted to TUs and were plotted to determine

the toxicant correlation to whole effluent toxicity (USEPA, 1989b). As shown in Figure A-2, more acute toxicity was present than would be explained by diazinon alone; the slope of the linear regression was less than 1 and all of the plotted data points are below the expected 1:1 relationship for diazinon and effluent toxicity. Spiking experiments also showed that doubling the concentration of diazinon in effluent samples did not result in a doubling of effluent toxicity. These results suggested that diazinon was not the sole cause of acute effluent toxicity.

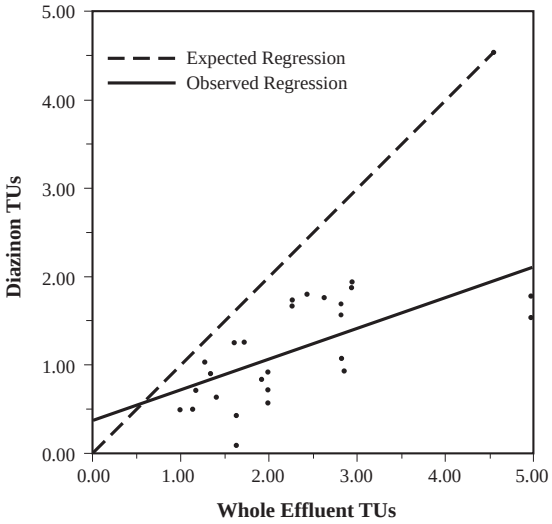

Figure A-2. Correlation of diazinon TUs versus whole effluent TUs (Source: USEPA, 1988).

TIE Procedure Update

The current approach (USEPA, 1993b) is to plot effluent TUs on the Y-axis (dependent variable) and toxicant TUs on the x-axis (independent variable). See Figure A-3.

Follow-up GC/MS analyses identified chlorfenvinphos (CVP) and malathion in effluent samples. Malathion did not appear in concentrations high enough to cause acute toxicity to *C. dubia*, although CVP concentrations were sufficient to contribute to effluent toxicity (48-hour LC50s of 1.4 and 0.35 µg/L, respectively, according to D. Mount, personal communication, USEPA, Duluth, Minnesota, 1989).

The correlation analysis was repeated using the summed toxic units for both diazinon and CVP versus whole effluent toxic units (USEPA, 1993b). As shown in Figure A-3, the slope of the regression line was close to 1, the y-intercept was nearly zero, and the r-value indicated a good correlation (r = 0.73). These results show that diazinon and CVP accounted for nearly all of the acute effluent toxicity.

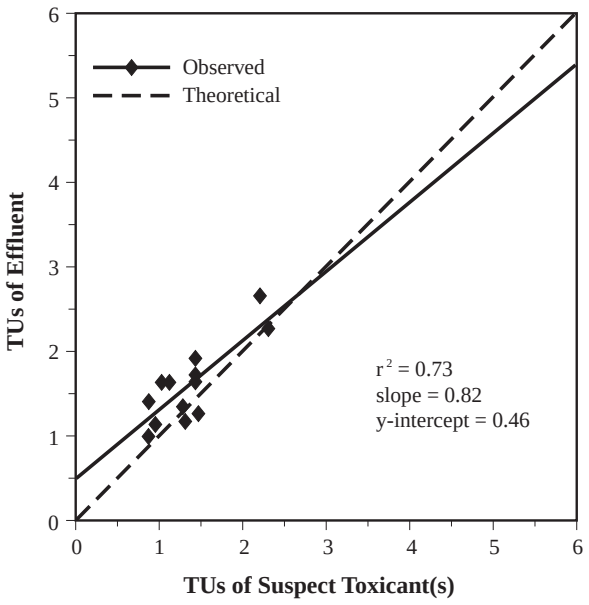

Figure A-3. Correlation of diazinon and CVP TUs versus whole effluent TUs (Source: USEPA, 1993b).

Additional confirmation testing involved analyzing 13 effluent samples using the C18 SPE column mass balance approach. As shown in Table A-1, in 12 of the 13 tests, the toxicity of all methanol fractions combined was slightly greater than the toxic fractions combined. Various mixtures of the three identified insecticides were tested to determine if interactive effects (i.e., antagonistic or synergistic) could account for the difference in toxicity. These tests showed that the toxicity of the insecticides was strictly additive. These results indicated that the higher toxicity of "all fractions" compared to the toxicity of the "toxic fractions" may be due to another unidentified toxicant, rather than interaction among the identified toxicants.

The additional toxicity observed in the "all fraction" test was attributed to 70% methanol/water fraction, which exhibited slight and intermittent toxicity. This fraction was initially included in the "nontoxic fraction" test; however, the mass balance approach

Table A-1. Comparison of Whole Effluent TUs and Methanol Fraction TUs

Sample	Acute Toxic Units (TUa)		
	Whole Effluent	All-Fractions	Toxic-Fractions
A	1.18	1.64	1.43
B	2.00	2.94	3.13
C	1.93	2.86	2.53
D*	<1.00	1.15	<1.00
E	2.00	1.75	1.64
F*	1.15	1.06	<1.00
G	1.33	1.52	1.13
H	3.70	3.03	2.86
I	2.86	2.86	2.44
J	2.27	1.72	1.64
K	2.27	2.04	2.00
L	2.27	1.67	1.59
Mean	2.13	2.18	2.00

* Values excluded from mean calculations due to less-than values.

indicated it to be a slightly toxic fraction. When the toxic units of the 70% fraction are added to the "toxic fraction" result, nearly all of the toxicity is accounted for. Due to the intermittent toxicity of this fraction, additional testing to identify the toxicant was not performed.

Additional species testing with *P. promelas* provided further evidence that the toxicants were organophosphate insecticides. No acute toxicity was observed with *P. promelas*, which are known to be orders of magnitude less sensitive to diazinon than *C. dubia* (USEPA 1987, 1988).

As a final confirmation step, the same symptoms to *C. dubia* were observed after exposure to effluent samples, toxic methanol fractions, and laboratory water spiked with near lethal levels of diazinon, CVP, and malathion. Similar symptoms were observed for all test solutions, which suggested that the same toxicant was responsible in each case.

Lawton, Oklahoma

The City of Lawton was required by USEPA Region 6 to initiate a TRE study in 1991, based on evidence of chronic effluent toxicity at its POTW (Engineering Science, Inc., 1991). The permit limit of no chronic lethality at the critical instream dilution of 96% (i.e., NOEC >96% effluent) was exceeded. Toxicity test results showed that the effluent was toxic to *C. dubia*, but not *P. promelas*.

TIE Phase I tests were conducted in 1991 to characterize the chronic effluent toxicants (Engineering Science, Inc., 1991). The permit limit was based on lethality to *C. dubia* and *P. promelas* in chronic toxicity tests; therefore, the TIE tests focused on lethality instead of reproduction or growth effects. The Phase I tests evaluated percent survival of *C. dubia*, the most sensitive organism, over 5 to 7 days in 100% effluent. In addition, acute lethality results (48- to 72-hour exposure) also were collected to assist in the evaluation.

The results indicated a consistent reduction in effluent toxicity by passing samples through the C18 SPE column. Chronic lethality data showed that no other treatment consistently removed toxicity. Toxicity was recovered by eluting the C18 SPE column with methanol, which indicated the presence of nonpolar organic toxicants. Sample adjustment to pH 3 and pH 11 also reduced toxicity in all but two samples, which suggested that the toxicants could be denatured under acidic or basic conditions.

TIE Procedure Update

As noted above, PBO, a metabolic blocker, can be added to toxic effluent samples, C18 SPE fractions, or HPLC fractions to test for the presence of metabolically activated toxicants such as organophosphate insecticides.

Reproduction data for C. dubia, although not required as part of compliance testing for the Lawton POTW, may have been useful in characterizing the effluent toxicants. These data may provide a more sensitive endpoint than survival in 100% effluent when comparing the effects of the various TIE treatments.

TIE Phase II tests were performed on three samples evaluated in the Phase I characterization and involved the following steps as described by USEPA (1989a):

- C18 SPE columns were eluted with a series of increasing methanol concentrations (25, 50, 75, 80, 85, 90, 95, and 100%) to isolate the toxicants.
- The acute toxicity of each eluted fraction was determined and the fractions found to be toxic were combined. The combined toxic fractions were then reconcentrated using a second C18 SPE

column. Acute toxicity tests were used instead of chronic toxicity tests because the methanol elution concentrated the toxicants to acutely toxic levels.
- The concentrated sample was separated into 30 fractions using HPLC and the toxicity of each fraction was measured. Again, the toxic fractions were combined and reconcentrated on another C18 SPE column.
- The combined toxic sample was then analyzed by GC/MS.

As shown in Table A-2, toxicity was consistently isolated in the 75 and 80% methanol fractions, although toxicity was also recovered in the 50% methanol fraction of one sample. Further separation of the toxicants by HPLC recovered toxicity in a relatively narrow band of fractions (fractions 22 to 28).

Table A-2. Summary of TIE Phase II Results

Sample	Sample Collection Data (1992)		
	4/28	6/11	7/16
C. dubia percent survival in 100% sample			
Original effluent	50	0	0
Post C18 SPE	100	100	80
SPE eluate (1× effluent)	0	0	0
Toxic methanol fractions (>20% mortality)			
Methanol/water (1× effluent conc.)	50% 75% 80%	75% 80%	75% 80%
HPLC fraction no. (1× effluent conc.)	15 22–25 30	25 28	22 24
Organophosphate insecticides in effluent (µg/L)			
Diazinon	0.22	0.42	0.71
Diazinon oxon	0.1	<0.1	1.45

GC/MS analysis of the toxic HPLC fractions identified several potentially toxic compounds, including the organophosphate insecticide, diazinon, and its metabolite, diazinon oxon (Table A-2). The 48-hour LC50 of diazinon to *C. dubia* is reported to range from 0.35 to 0.61 µg/L (Amato et al., 1992; Ankley et al. 1991). Based on the low end of this range, the diazinon concentrations in the Lawton effluent were high enough to cause acute toxicity to *C. dubia* in two of the three samples tested (0.42 and 0.71 µg/L for the June and July samples, respectively).

C. dubia acute toxicity tests were conducted to evaluate the potential contribution of diazinon oxon to effluent toxicity. The 48-hour LC50 for diazinon oxon was determined to be 1 µg/L. These data indicate that the diazinon oxon concentration in the July effluent sample (1.45 µg/L) was high enough to contribute to the observed acute toxicity.

TIE Procedure Update

Data on the chronic toxicity of organophosphate insecticides is limited. Unpublished data (TRAC Laboratories, 1992) suggest that *C. dubia* may be chronically sensitive to 0.12 to 0.38 µg/L diazinon (see also Section 2). Chronic data would have been useful in defining the potential for diazinon to contribute to chronic toxicity at the Lawton POTW.

Further testing focused on confirming the contribution of diazinon and diazinon oxon to effluent toxicity. A partial Phase III confirmation was performed using the following steps (USEPA, 1989b):

- Assessing diazinon's physical/chemical properties in relation to the TIE results.
- Determining the contribution of diazinon and diazinon oxon to whole effluent toxicity based on measured effluent concentrations.
- Reviewing effluent toxicity data for a 3-year period to determine if the occurrence of effluent toxicity matched seasonal insecticide use (Engineering Science, Inc., 1992).

Diazinon matches the general toxicant profile developed as part of the TIE. Removal of diazinon on the C18 SPE column and its elution at high methanol concentrations is consistent with diazinon's characteristic as an organic chemical of low polarity. The observed reduction in toxicity by pH adjustment also is indicative of diazinon's tendency to break down under acidic and alkaline conditions.

Concentrations of diazinon and diazinon oxon were measured in 13 effluent samples collected from April 1 through August 21, 1992. Chronic toxicity data for the insecticides were not available at the time; therefore, it was not possible to apply the correlation approach. However, in seven cases, diazinon exceeded the 0.35 µg/L acute toxicity value for *C. dubia*. In two of these cases, diazinon oxon concentrations also exceeded the acute toxicity value of 1.45 µg/L. These

data suggested that diazinon and diazinon oxon were likely to cause mortality equal to or greater than that found in the effluent samples.

A review of effluent toxicity data from 1989 to 1992 indicated a greater incidence of toxicity in the spring and summer of each year when insecticides are most often used. Effluent toxicity decreased in late summer and fall and generally disappeared in the winter months. These data support the evidence that toxicity is associated with insecticides.

TIE Procedure Update

Confirmation of the role of diazinon and other toxicants would have been more definitive if the current Phase III procedures (USEPA, 1993b) for chronic toxicants had been applied. Useful procedures for confirming organophosphate insecticide toxicity include the correlation, mass balance, and species symptoms approaches. An example of the use of these procedures is presented in Appendix F.

Based on previous studies (City of Greenville, 1991; C. Kubula, personal communication, City of Greenville, Texas, Public Works Department, 1992), the City of Lawton decided to implement a public awareness program in 1993 to control the discharge of insecticides to the POTW. Information on the proper use and disposal of insecticides was printed in newspaper articles and on monthly water bills (Engineering-Science, Inc., 1993). An electronic message sign with insecticide information was also located at major intersections. Since August 1993, the POTW effluent has met the toxicity permit limit (NOEC >96% effluent) with the exception of 2 months in 1994 and several months in 1995 (as of September 1997). Although diazinon was not confirmed as an effluent toxicant, the City's ongoing insecticide control effort appears to have been successful in achieving compliance with the chronic toxicity limit.

Akron, Ohio

A survey of six Ohio municipal wastewater treatment plants was conducted to determine the level of toxicity reduction that can occur in POTWs (Neiheisel et al., 1988). Of the six WWTPs, the City of Akron's Botzum WWTP received the most toxic influent wastewater. Significant toxicity reduction was achieved through treatment; however, the effluent had an impact on the Cuyahoga River. A bioassessment study of the river in 1984 revealed a severe impairment to aquatic communities downstream of the WWTP discharge. A review of the WWTP's operating records showed a history of intermittent bypasses of raw wastewater during storm events.

Based on the survey results, the Botzum WWTP was selected by USEPA as a site for a TRE research study. The research study focused on conducting toxicity tests of the effluent and the bypassed wastewater and characterization of the variability and sources of the impairment to the receiving water (Mosure et al., 1987). In addition, TIE tests were performed to try to identify the effluent toxicants.

Toxicity test results indicated that although CSOs may contribute intermittently to poor river quality, the continuous effluent discharge was probably the major cause of the observed impact (Mount and Norberg-King, 1985).

The TIE testing isolated toxicity on the C18 SPE column and the toxicity was eluted in the 85% methanol/water fraction (Mosure et al., 1989). These results suggested that non-polar organic compounds were a principal cause of effluent toxicity. Metals also were implicated as effluent toxicants. However, before toxicant identification and confirmation could be performed, effluent toxicity abated.

The cause of this abatement is not known, although the following events may have contributed to the improved effluent quality. These events include:

- Increasing MLSS concentrations in the WWTP aeration basins.
- The shutdown of a large chemical manufacturing plant that discharged to the WWTP.
- Overall improvements in WWTP operation and the pretreatment program (Mosure et al., 1987).

Biological surveys of the Cuyahoga River in 1986 continued to show poor water quality despite the decrease in effluent toxicity (Mosure et al., 1987). It is possible that other dischargers to the river were contributing to the impairment or the recovery rate of the river was slower than anticipated.

Billerica, Massachusetts

A study was conducted at the City of Billerica's WWTP to evaluate sources of toxicity in the facility's

Toxicity Control Evaluation Update

Abatement of effluent toxicity during the course of TREs is not uncommon. However, efforts to ensure ongoing compliance can be difficult when the original causes and sources of toxicity are not known. These situations dramatize the importance of documenting industrial pretreatment activities and POTW operations in the early stages of the TRE. Weekly or daily reports of production and waste discharge activities by industrial users can provide a useful history of events that can be used to indicate potential sources. This information is also helpful in subsequent pretreatment control studies if an industrial user is identified as a source of toxicity (Botts et al., 1994).

collection system (Durkin et al., 1987). A purpose of the study was to evaluate the usefulness of Microtox® as a tool for tracing sources of toxicity.

The Billerica study was conducted in five stages:

- Screening for WWTP influent toxicity.
- Testing samples from pump stations in the collection system.
- In-depth testing to determine the time of day when toxicity was observed at the pump stations.
- Testing of the main sewer lines above the pump stations where toxicity was indicated.
- Final testing of tributary sewers.

Of the 11 pump stations tested, 2 were found to have highly toxic wastewaters. In one of these pump stations, high levels of toxicity occurred only during the 8 a.m. to 2 p.m. time period. Further investigation of the intermittently toxic pump station provided evidence that the principal source of toxicity was an industrial park.

RTA Procedure Update

Toxicity screening tools such as Microtox® have been used to identify sources of toxicity in POTW collection systems. It is necessary to first determine if a correlation exists between the compliance test and the screening test to ensure that the toxicity measured by the surrogate tool is the same toxicity indicated by the species used for compliance testing. This correlation can be performed using POTW effluent; however, it is important to note that correlation results may be different for individual industrial discharges. As a result, the screening test may yield false positive or false negative results.

The advantage of screening tests is that a large number of samples can be processed at relatively low cost. As an alternative to these tools, POTW staff may consider using the permit test species in an abbreviated test procedure such as that used in the TIE (USEPA 1991). The cost of these tests can be comparable to commercially available screening tests if the number of replicates or sample concentrations is reduced or the exposure time is decreased.

Although this study indicated a potential source of toxicity, a final determination of the source(s) of toxicity would require first treating the sewer samples in a simulation of the POTW to provide an accurate estimate of the refractory toxicity of the waste stream. Otherwise, as discussed in Section 5, the toxicity results may overestimate the toxicity of the discharge because some toxicity removal generally occurs in the POTW. A description of the updated RTA protocol is given in Section 5.

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